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Biography:

Carlos A. Castañeda is an assistant professor of Biology and Chemistry at Syracuse University. He received his PhD in Molecular Biophysics from Johns Hopkins University working with Dr. Bertrand García-Moreno on the electrostatic energies of proteins. He completed his postdoctoral work with David Fushman at the University of Maryland-College Park. There, he entered the ubiquitin field studying the structure and function of all polyubiquitin chain types using NMR spectroscopy, small angle scattering, and computational modeling. He received a NSF Postdoctoral fellowship while at UMD. Since starting at Syracuse University in 2014, Carlos has focused on elucidating the role of protein quality control mechanisms in neurological disorders, such as amyotrophic lateral sclerosis (ALS). The lab studies ubiquilin (UBQLN) proteins, particularly UBQLN2, an ALS-linked protein. His lab recently discovered that UBQLN2 is recruited to stress granules, membraneless organelles that are hypothesized to form by liquid-liquid phase separation. Importantly, the lab also demonstrated that UBQLN2 phase separates in vitro. For this work, Carlos has received two grants from the ALS Association, and a NSF CAREER award in 2018. His lab's recent work was published in Molecular Cell.

Abstract:

"Protein droplets and ALS: Liquid-liquid phase separation of UBQLN2 is modulated by oligomerization, ALS-linked mutations, and ubiquitin binding"

An emerging feature of many intrinsically-disordered proteins is that they demix from solution and form a protein-dense phase (liquid droplets) in equilibrium with a protein-dilute phase, a phenomenon generally known as liquid-liquid phase separation (LLPS). LLPS underlies the formation of biomolecular condensates such as stress granules. Stress granule persistence or disrupted stress granule dynamics is hypothesized to lead to the characteristic protein inclusions that are a hallmark of ALS (amyotrophic lateral sclerosis) and other neurological disorders. We recently found that Ubiquilin-2 (UBQLN2), an ALS-linked protein critical for maintaining protein quality control, is recruited to stress granules in vivo and undergoes LLPS in vitro under physiological conditions. UBQLN2 LLPS behavior is modulated by multivalent interactions involving its folded domains as well as the intrinsically-disordered STI1-II and proline-rich (Pxx) regions. Importantly, we showed that binding to ubiquitin or polyubiquitin eliminates UBQLN2 phase separation; this has potential implications for the role of UBQLN2 in shuttling ubiquitinated substrates to the proteasome or autophagy pathways. Mutations in the (Pxx) region of UBQLN2 cause ALS and ALS/dementia, but the molecular mechanisms for how these mutations cause disease are unknown or poorly understood. Since the Pxx region contributes to UBQLN2 LLPS, we hypothesized that Pxx mutations disrupt LLPS. Using spectrophotometric assays and microscopy, we show that a subset of these mutations at positions T487, P497 or P506 significantly enhance UBQLN2 LLPS and/or alter material properties of UBQLN2 protein droplets in vitro. Importantly, these UBQLN2 mutants still undergo LLPS reversibly. Biophysical experiments including NMR spectroscopy and analytical ultracentrifugation reveal that these single point mutations do not alter UBQLN2 structure, but likely promote UBQLN2 oligomerization, a prerequisite for LLPS. Our experiments suggest that disease-linked mutations modulate UBQLN2 LLPS, and potentially alter material properties of UBQLN2-containing biomolecular condensates in the cell, promoting disease states.